Feedback effects between plant and flower-visiting insect communities along a primary succession gradient

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Abstract: Primary successions of glacier forelands are unique model systems to investigate community dynamics and assembly processes. However, successional changes of plant and insect communities have been mainly analysed separately. Therefore, changes in plant–insect interactions along successional gradients on glacier forelands remain unknown, despite their relevance to ecosystem functioning. This study assessed how successional changes of the vegetation influenced the composition of the flower-visiting insect assemblages of two plant species, Leucanthemopsis alpina (L.) Heyw. and Saxifraga bryoides L., selected as the only two insect-pollinated species occurring along the whole succession. In addition, we investigated the links between reproductive output of these plants and pollinator abundance through experimental exclusion of pollinators. Plant community structure changed along the succession, affecting the distribution and the abundance of insects via idiosyncratic responses of different insect functional groups. L. alpina interacted with ubiquitously distributed pollinators, while S. bryoides pollinators were positively associated with insect-pollinated plant species density and S. bryoides abundance. With succession proceeding, insect assemblages became more functionally diverse, with the abundance of parasitoids, predators and opportunists positively related to an increase in plant cover and diversity. The reproductive output of both plant species varied among successional stages. Contrary to our expectation, the obligate insect-pollinated L. alpina showed a reproductive output rather independent from pollinator abundance, while the reproductive output of the self-fertile S. bryoides seemed linked to pollinator abundance. Observing ecological interactions and using functional traits, we provided a mechanistic understanding of community assembly processes along a successional gradient. Plant community diversity and cover likely influenced insect community assembly through bottom-up effects. In turn, pollinators regulate plant reproductive output through top-down control. We emphasise that dynamics of alpine plant and insect communities may be structured by biotic interactions and feedback processes, rather than only be influenced by harsh abiotic conditions and stochastic events.

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Feedback effects between plant and flower-visiting insect communities along a primary succession gradient

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Abstract

Primary successions of glacier forelands are unique model systems to investigate community dynamics and assembly processes. However, successional changes of plant and insect communities have been mainly analysed separately. Therefore, changes in plant–insect interactions along successional gradients on glacier forelands remain unknown, despite their relevance for ecosystem functioning. This study assessed how successional changes of the vegetation influenced the composition of the flower-visiting insect assemblages of two plant species, *Leucanthemopsis alpina* (L.) Heyw. and *Saxifraga bryoides* L., selected as the only two insect-pollinated species occurring along the whole succession. In addition, we investigated the links between reproductive output of these plants and pollinator abundance through experimental exclusion of pollinators. Plant community structure changed along the succession, affecting the distribution and the abundance of insects via idiosyncratic responses of different insect functional groups. *L. alpina* interacted with ubiquitously distributed pollinators, while *S. bryoides* pollinators were positively associated with insect-pollinated plant species density and *S. bryoides* abundance. With succession proceeding, insect assemblages became more functionally diverse, with the abundance of parasitoids, predators and opportunists positively related to an increase in plant cover and diversity. The reproductive output of both plant species varied among successional stages. Contrary to our expectation, the obligate insect-pollinated *L. alpina* showed a reproductive output rather independent from pollinator abundance, while the reproductive output of the self-fertile *S. bryoides* seemed linked to pollinator abundance. Observing ecological interactions and using functional traits we provided a mechanistic understanding of community assembly processes along a successional gradient. Plant community diversity and cover likely influenced insect community assembly through bottom-up effects. In turn, pollinators regulate plant

reproductive output through top-down control. We emphasize that dynamics of alpine plant and insect communities may be structured by biotic interactions and feedback processes, rather than only be influenced by harsh abiotic conditions and stochastic events.

Keywords: community assembly, functional traits, glacier forelands, plant–insect interactions, reproductive output

Introduction

Glacier forelands are areas in front of retreating glaciers (Matthews 1992). They are the product of the deglaciation since the end of the Little Ice Age (1500-1850 AD). Glacier forelands represent unique model systems for studying ecosystem development (Walker et al. 2010) and community assembly processes along gradients of primary successions (Foster and Tilman 2000). Following the time-sequence approach, it is assumed that plots of different time since deglaciation represent different community development stages along a successional gradient (Walker et al. 2010).

Successional changes of plant and insect communities were historically described separately. Studies of primary succession along glacier forelands mainly focused on plant colonization patterns (Matthews 1992; Erschbamer et al. 2008), on mechanisms of plant succession related to environmental changes (Caccianiga et al. 2001; Hodkinson et al. 2003), on plant species reproductive features (Erschbamer et al. 2001; Raffl et al. 2007; Jones and del Moral 2009), and on plant functional traits (Caccianiga et al. 2006; Erschbamer and Mayer 2011). From a zoological point of view, studies mainly focused on diversity patterns of epigean arthropod succession (e.g. Kaufmann 2001; Gobbi et al. 2006; Vater and Matthews 2013) and on community-environment relationships (e.g. Hodkinson et al. 2001; Gobbi et al.

2007).

However, little attention is given to plant–insect interactions along glacier foreland successions (Albrecht et al. 2010), despite their role in governing community dynamics (Losapio et al. 2015). Few recent studies showed common patterns in plant and ground beetle functional traits (Gobbi et al. 2010), revealed the structure of plant–pollinator networks (Albrecht et al. 2010; Losapio et al. 2015) and examined food web properties (König et al. 2011; Raso et al. 2014).

Despite the pivotal role played by plant–insect interactions for structuring communities (Scherber et al. 2010), whether vegetation changes may influence the assembly of flower-visiting insect communities along primary succession remains unexplored. Furthermore, knowledge about the links between pollinator abundance and plant reproductive output along primary succession gradients is still missing.

In the present study we aimed to describe feedbacks effects between plant and insect communities along the primary succession of a glacier foreland (Vedretta d'Amola debris-covered glacier, Adamello-Presanella Alps, Italy). In particular, we investigated how vegetation changes along the succession influenced the composition of the flower-visiting insect assemblages of two plant species, *Leucanthemopsis alpina* (L.) Heyw. and *Saxifraga bryoides* L. In addition, we assessed the breeding systems (i.e. cross- vs self-pollination) of these species and the links between plant reproductive output (i.e. number of fruits and seeds) and pollinator abundance. Along the successional gradient we analysed: (i) changes in plant cover, species diversity, species evenness, and plant pollination strategy; (ii) the distribution and the abundance of insect assemblages on *L. alpina* and *S. bryoides* and their relationship with the surrounding plant community structure, and (iii) the links between *L. alpina* and *S. bryoides* reproductive output and pollinator abundance. Overall, we hypothesised that

97 successional changes of the vegetation can influence the functional composition of the flower-
98 visiting insect assemblages. In turn, we expected a link between plant reproductive output and
99 pollinator abundance.
100
101 **Materials and methods**
102 **Study area**
103 The study was performed along the primary succession of Vedretta d'Amola debris-covered
104 glacier foreland (Adamello-Presanella Alps, Italy, 46°13'16"N, 10°40'41"E). This glacier
105 foreland is 1.23 km long, facing south-east and extending from 2425 to 2560 m a.s.l. (Online
106 Resource Fig. 4). The glacier tongue position during the three main advance phases was dated
107 back to the end of the Little Ice Age (*c.* 1850 A.D.), 1925 A.D. and 1994 A.D. The nearest
108 climatic station in Pradalago at 2084 m a.s.l., about 9 km north-east from the study site,
109 receives a mean annual rainfall of 1395 mm and mean annual temperate is 2.6 °C. Snow cover
110 usually lasts from October to July. The glacier foreland has a patchy vegetation (*c.* 10-70%
111 plant cover) characterised by *Geum reptans* L.*, Oxyria digyna* (L.) Hill*,* and *Luzula alpino-*
112 *pilosa* (Chaix) Breistr. Outside the glacier foreland, the vegetation is dominated by *Carex*
113 *curvula* All. and *Salix herbacea* L. (Online Resource Tab. 1).
114
115 *Study species*
116 We studied plant–insect interactions between the flowering plants *Leucanthemopsis alpina*
117 (L.) Heyw. (Asteraceae) and *Saxifraga bryoides* L. (Saxifragaceae) and their flower-visiting
118 insects. These two focal plant species were selected as they were the only two insect-
119 pollinated species occurring along the whole successional gradient (Online Resource Tab.1).
120 *L. alpina* is a perennial hemicryptophyte, forming leaf rosettes with prostrate stems and

121 deeply incised leaves (Online Resource Fig. 7). Reproductive shoots usually bear one terminal
122 capitulum (hereafter, inflorescence) consisting of yellow disk florets surrounded by white
123 ligulate ray florets. The fruit is an achene devoid of pappus. The species inhabits scree slopes,
124 grasslands and snowbeds from the subalpine to the nival zone (Pignatti 1982).
125 *S. bryoides* is a perennial semi-evergreen cushion plant forming densely matted shoots
126 with rigid, recurved leaves (Online Resource Fig. 8). Reproductive shoots usually bear one
127 terminal hermaphroditic white-yellowish flower with nectaries. The fruit is a bi-locular
128 capsule containing hundreds of tiny seeds. The species prefers siliceous substrates and grows
129 mainly in open habitats on scree and solid rock, from the alpine zone up to the nival zone
130 (Ladinig and Wagner 2007).
131
132 **Study design**
133 Along the successional gradient, five sites corresponding to the following successional stages
134 were selected: successional stage 0 (the debris-covered glacier surface), successional stage I
135 (1-20 years), successional stage II (21-90 years), successional stage III (91-160 years),
136 successional stage IV (>160 years). Each sampling site included two 25 m² plots (5m x 5m),
137 one for *L. alpina* and one for *S. bryoides*. Plots were established in areas not subjected to
138 disturbance.
139
140 *Sampling protocol*
141 Vascular plant species and bryophytes occurring within each 25 m² plot were recorded. The
142 overall plant cover and the abundance of every plant species were visually estimated with a
143 resolution of 5% (Online Resource Tab.1). Nomenclature follows Aeschimann et al. (2004).
144 For each plot, flower-visiting insects were collected during the 2012 and 2013 flowering

145 seasons (July and August). Flower-visiting insects were sampled with an entomological
146 aspirator, according to the time-observation plot method (Gibson et al. 2011; Nielsen et al.
147 2011). Within each plot, three sampling units were selected for each plant species, consisting
148 of an *L. alpina* tuft or an *S. bryoides* cushion, respectively (size about 20 cm diameter). The
149 three sampling units were surveyed together during periods of 40 minutes. Sampling was
150 repeated at 11am, 1pm, 3pm randomly across the season, resulting in 9 samples collected for
151 each plot at each successional stage every year. Due to long-lasting snow during 2013, *S.*
152 *bryoides* did not flower at the successional stage 0, while at stages I, II and III we collected 6
153 samples each. A total of 90 (45 for *L. alpina* and for *S. bryoides,* each) and 72 (45 and 27 for
154 *L.alpina* and *S. bryoides*, respectively) samples were carried out during 2012 and 2013,
155 respectively (Online Resource Tab. 2).

156 During each sampling occasion, the following weather conditions were recorded: (i) air
157 temperature and wind speed, at 0.5m above ground with a digital therm-anemometer (NTC
158 Thermometer Anemometer, ETANG Electronics, Birmingham, UK); (ii) cloud cover, visually
159 estimated with a resolution of 10% (100% as clear sky), and (iii) presence of gusts of wind
160 and/or rainfall (weather conditions during sampling reported in Online Resource Tab. 2). To
161 not undermine insect activity, we sampled from a distance of 0.5m away from plants and we
162 wore dull clothes.

163 Flower-visiting insects were identified to species level, when possible, otherwise to
164 genus or family level. Insect taxa were categorised into six functional groups, according to
165 their trophic guilds obtained form the literature: detritivores, phytophagous, pollinators,
166 predators, parasitoids, and opportunists (Mellini 1997; Gregor et al. 2002; Oosterbroek 2006;
167 Jedlička et al. 2009; Gobbi and Latella 2011, Speight 2013). Insect nomenclature follows de
168 Jong (2013).

169 To determine plant breeding systems (i.e. cross- vs self-pollination) and plant
170 reproductive output (i.e. number of fruits or seeds), a breeding experiment was conducted for
171 paired samples along the succession during 2013. Within each plot, five individuals per plant
172 species were randomly selected. For each individual, before anthesis one inflorescence/flower
173 was bagged with a closed fine-meshed nylon bag (hereafter treatment), while another
174 inflorescence/flower was bagged with an open bag (hereafter control). At the end of anthesis,
175 a closed bag was fixed on the control flower. Bags (n = 92; Online Resource Tab. 3) were
176 harvested in early October. Bags were stored at room temperature for 72h in paper before
177 fruits and seeds were counted.
178
179 **Statistical analysis**
180 *Plant community structure*
181 In order to examine how successional changes of the vegetation can influence the composition
182 of flower-visiting insect assemblages on the focal plant species, we first examined changes in
183 plant community structures along the succession. Thus, the following variables supposed to
184 describe successional changes in biodiversity and biomass of vegetation (Walker et al. 2010)
185 were considered to describe the plant community structure: plant cover, Shannon diversity,
186 Shannon evenness, insect-pollinated plant density and insect-pollinated plant richness. Insect-
187 pollinated plant density (e.p.d.) represents the relative abundance of insect-pollinated plant
188 species, while insect-pollinated plant richness (e.p.r.). represents the relative richness of
189 insect-pollinated plant species. Specifically:

$$e.p.d. = \sum_{i=1}^{P} c_i \times \sigma_i$$

$$e.p.r. = \frac{\sum_{i=1}^{P} s_i \times \sigma_i}{P}$$

where for the plant species i in a community with P species, c is the relative abundance (i.e. relative cover) of the species i, s is species presence (i.e. presence/absence), σ is the pollination strategy, with $\sigma = 1$ for insect-pollinated species and $\sigma = 0$ for wind-pollinated species. These metrics have values ranging from 0 to 1. When a plant community is entirely composed by insect-pollinated plants, e.p.d. and e.p.r. are equal to 1. Vice versa, when a plant community is entirely composed by wind-pollinated plants, e.p.d. and e.p.r. are equal to 0.

To analyse the multivariate relationships among ecological indices of plant community structure, a Principal Component Analysis (PCA) was carried out. Then, ANOVA-GLMs were used to examine differences among successional stages (response, categorical variable with five classes) for each ecological index (explanatory, continuous variables).

Flower-visiting insect assemblages

To assess differences of total insect abundance among successional stages, GLMs (Poisson errors) were used, with insect abundance as response and successional stage as explanatory variable. Separated analyses were performed for *L. alpina* and *S. bryoides*. Then, the flower-visiting insect assemblages was examined focusing both on taxonomical and functional composition (i.e. the six insect functional groups mentioned above). To examine taxonomic and functional diversity changes along the succession, the Shannon index (at family level) was calculated by pooling data for each successional stage. Pooled data were then plotted along the successional gradient.

To describe the variation in insect assemblages along the successional gradient as well as the associations among insect abundances and plant community structure, partial Canonical Correspondence Analyses (pCCA) (ter Braak 1986; Jongman et al. 1995) were carried out. Separated analyses were performed for *L. alpina* and *S. bryoides* assemblages, considering as

response variable the abundance of insects, at both taxonomical and functional levels. Thus, in total four pCCAs were performed pooling insect abundance for each sampling unit (i.e. plant tuft or cushion) for the two sampling years. Taxa and functional roles with a total abundance lower than 5% were excluded. Thus, for the pCCAs using the taxonomical information of insects, Coleoptera, Staphylinidae, Diptera Anthomiidae, Muscidae, Syrphidae and Hymenoptera Ichneumonidae families were examined. For the pCCAs using the functional groups of insects, pollinators, predators, opportunists, phytophagous and parasitoids were considered. The explanatory 'environmental' variables were plant cover, Shannon index, Shannon evenness, e.p.d. and e.p.r. The cover and the number of *L. alpina* inflorescences and *S. bryoides* flowers were included as covariates, as they may potentially influence flower visitors activity (Potts et al. 2003; Hegland and Boeke 2006). For all explanatory variables a preliminary assessment of correlation was carried out (using the Pearson correlation coefficient) in order to minimize multicollinearity issues in the pCCAs. When the correlation between variables exceeded 0.75, only one of these variables was included in the analysis, the choice based on subject-matter considerations (Legendre and Legendre 2012). Since discontinuities in the sample distribution of plant cover and number of *L. alpina* inflorescences were revealed, before performing the pCCAs these variables were categorized as follows: plant community cover in two classes (<50, >50) and number of *L. alpina* inflorescences in three classes (<10, 20-25, >30). Weather conditions during sampling (mean temperature, wind speed, cloud cover and presence of strong wind gusts) were considered as potential confounding effects, and included in the pCCAs as conditioning variables (Oksanen et al. 2014). Results were reported in terms of proportions of explained inertia as well as plotting the pCCA diagram.

Plant breeding systems

To identify the breeding systems of *L. alpina* and *S. bryoides*, seed set was analysed considering: (i) Reproductive potential: number of ovaries per *L. alpina* inflorescence and number of ovules per *S. bryoides* flower, and (ii) Reproductive output: number of *L. alpina* fruits and number of *S. bryoides* seeds divided by reproductive potential. To assess differences in reproductive potential and reproductive output between treatment and control bags, conditional logistic regression models were used (Thernau 2014). Such a model allowed us to account for the 'pairing' between treatment and control bags within the same plant. Results were reported in terms of conditional odds ratio.

Finally, to link the reproductive output of *L. alpina* and *S. bryoides* with pollinator abundance, the mean plant reproductive output the pollinator abundance (for the 2013 season) were plotted together within each successional stage.

All statistical analyses were performed in R version 3.0.2. (R Core Team 2014).

Results

Plant community structure

Plant community structure varied along the successional gradient (Fig. 1). Sixty percent of the variation was represented by the first principal component. This axis run from stage 0-I communities, which had low plant cover (*c.* 10%), low Shannon diversity (2-2.4), high e.p.d. (0.7-0.9), and high evenness (0.9-1), to stage III-IV communities, which had high plant cover (*c.* 80-90%), high Shannon diversity (2.5-3), low e.p.d. (0.4-0.6), and low evenness (0.8-0.9).

Plant cover, Shannon diversity, evenness, and e.p.d. significantly varied among successional stages (ANOVA-GLM χ^2-test: $p < 0.001$ for each variable), while e.p.r. did not show any significant variation (Table 1).

262

263 **Flower-visiting insect assemblages**

264 Within the recorded plant–insect interactions, we collected 474 specimens on *L. alpina*, 97
265 and 377 during 2012 and 2013 (Online Resource Tab. 5), respectively, and 440 specimens on
266 *S. bryoides* 197 and 243 during 2012 and 2013 (Online Resource Tab. 6), respectively.
267 Diptera, Hymenoptera and Coleoptera were the most abundant orders, represented by 21, 13
268 and 6 families, respectively (Online Resource Tab. 7).

269 The insect assemblage on *L. alpina* was dominated by anthomyiid flies (Diptera:
270 Anthomyiidae) and house flies (Diptera: Muscidae) with 45% and 34% of all insects,
271 respectively. Rove beetles (Coleoptera: Staphylinidae) and hoverflies (Diptera: Syrphidae)
272 were less abundant, both with 6%. The insect assemblage on *S. bryoides* was characterised by
273 house flies with 23%, anthomyiid flies with 18%, rove beetles with 16% and ichneumonids
274 wasps (Hymenoptera: Ichneumonidae) with 14%. Considering insect functional roles,
275 pollinators represented 84% of all insects interacting with *L. alpina*, while predators and
276 opportunists represented 9% and 5% of the assemblage, respectively. On *S. bryoides* 59%
277 were pollinators, 15% parasitoids, 14% opportunists and 5% phytophagous.

278 The abundance of flower-visiting insects significantly changed among successional
279 stages (ANOVA-GLM χ^2-test: $p < 0.001$) both for *L. alpina* (Fig. 2a) and *S. bryoides* (Fig. 2b).
280 Furthermore, for both plant species, both the insect taxonomical and functional diversity
281 increased along the successional gradient (Fig. 2 c, d).

282 The variation in insect assemblages along the successional gradient in association with
283 plant community structure is presented in Fig. 3. Concerning the occurrence and the
284 abundance of insects interacting with *L. alpina* (Fig. 3a), weather conditions during sampling,
285 above all air temperature and cloud cover, explained 43.7% of the variation, while plant

286 community structure explained 28.9%. The first axis of the pCCA correlated mainly with
287 e.p.r. (r = 0.74) and e.p.d. indices (r = 0.40). Higher abundance of Staphylinidae and
288 Syrphidae was negatively associated with the first axis (weighted average scores = -1.31 and -
289 0.53, respectively), i.e. with low e.p.r. and low e.p.d. values. Anthomyiidae and Muscidae did
290 not show any specific association with environmental variables, mainly due to their
291 ubiquitous distribution along the succession (weighted average scores for axis 1 = 0.00 and
292 0.28, respectively).

293 Concerning the occurrence and the distribution of insects interacting with *S. bryoides*
294 (Fig. 3b), weather conditions during sampling explained 21.5% of the variation, while plant
295 community structure explained 59.0%. The first axis of the pCCA positively correlated with
296 plant cover (r = 0.77) and negatively correlated with e.p.d. (r = -0.89) and *S. bryoides* cover (r
297 = -0.72). Higher abundance of Ichneumonidae was positively associated with the first axis
298 (weighted average scores = 1.30), i.e. with high cover and both low e.p.r. and *S. bryoides*
299 abundance. Anthomyiidae and Staphylinidae showed an opposite trend (weighted average
300 scores for axis 1 = -0.46 and -0.44, respectively). A differentiation between successional stage
301 IV and all other stages emerged, being the former characterised by higher abundance of
302 Ichneumonidae.

303 Regarding the insect functional role distribution on *L. alpina* (Fig. 3c), weather
304 conditions during sampling explained 6.8% of variation, while plant community structure
305 explained 40.6%. The first axis was positively correlated with the e.p.r. index (r = 0.81) and
306 negatively correlated with a high abundance of *L. alpina* inflorescences (r = -0.66) and plant
307 cover (r = -0.61). Predators and opportunists were negatively associated with the first axis
308 (weighted average scores = -0.73 and -0.93, respectively), i.e. they were positively associated
309 with high plant cover and high *L. alpina* inflorescence abundance. Pollinators showed a

central position close to the axis origin (weighted average scores for axis 1 = 0.12). No evident clustering of sites was found in both analyses.

Concerning the insect functional role distribution on *S. bryoides* (Fig. 3d), weather conditions during sampling and plant succession variables explained 10.6% and 60.8% of the variation, respectively. The first axis was positively correlated with plant cover (r = 0.83) and negatively correlated with both e.p.d. (r = -0.90) and *S. bryoides* cover (r = -0.76). Pollinators were positively associated with low plant cover, high e.p.d. and high *S. bryoides* cover (weighted average scores for axis 1 = -0.42). On the other hand, parasitoids and opportunists were positively associated with the first axis (weighted average scores = 1.04 and 0.60, respectively), i.e. with high plant cover, low e.p.d. and low *S. bryoides* cover. The ordination of successional stages reflected the main topographical sequence of the study sites. Stages 0 and I were characterised by high relative abundance of pollinator comparted to parasitoids and opportunists. On the other hand, stage IV was characterised by high plant cover, high abundance of parasitoids and opportunists, and low pollinator abundance.

Plant breeding system

In total, 48 *L. alpina* inflorescences were collected and 3208 and 3391 ovaries were counted for treatment and control bags, respectively. In total, 44 *S. bryoides* flowers were collected and 2105 and 6017 ovules were counted for treatment and control bags, respectively (Online Resource Tab. 8). The reproductive potential of *L. alpina* did not significantly change between treatment levels (conditional odds ratio: 0.973; p = 0.101). For *S. bryoides* flowers, a significant influence of bag treatment on the reproductive potential was found (conditional odds ratio: 0.988; p < 0.001).

For *L. alpina,* the treatment effect on reproductive output was strong (p < 0.001), as

bagged inflorescences never produced fruits while control inflorescences produced on average 0.30 (±0.22 SD) fruits per ovary (Online Resources Fig. 5a). For *S. bryoides*, the reproductive output did not significantly change bag treatment levels (conditional odds ratio: 0.178; p = 0.140). Indeed, *S. bryoides* produced on average 0.16 (±0.30 SD) and 0.29 (±0.32 SD) seeds per ovary for treatment and control bags, respectively. (Fig. 5b).

The mean reproductive output of *L. alpina* decreased along the succession towards early successional stages, while its pollinator abundance increased (Online Resource Fig. 6a). The reproductive success of *S. bryoides* and its pollinator abundance showed a coupled trend among the successional stages, with an increase from stage 0 to stage II, followed by a decrease from stage II to stage IV (Online Resource Fig. 6b).

Discussion

Plant community structure

The prevailing pollination strategy of plant communities changed from insect-pollinated to wind-pollinated with succession proceeding, along with an increase in plant cover and plant diversity. Specifically, the debris-covered glacier and the early successional stages were characterised by scattered pioneer vegetation, which showed high evenness and a prevalent pollination strategy dependent on insects. Wind-pollinated species increased in late-successional stages, probably due to the increasing dominance of graminoids, which was linked to an increase in plant cover.

Several studies on primary succession along glacier forelands showed that plant cover (Matthews 1992), plant diversity (Caccianiga et al. 2001), community composition (Hodkinson et al. 2003), functional traits (Caccianiga et al. 2006; Erschbamer and Mayer, 2011), and facilitation for recruitment and establishment (Erschbamer et al. 2008) change

along successional gradients. In the present study, we showed that also plant pollination strategies changed along the succession. As a consequence, the observed change from prevailing insect-pollinated to wind-pollinated plant species may produce cascading effects on the assembly of insect community.

Flower-visiting insect assemblages

Pollinating flies were the dominant groups of insects visiting *L. alpina* along the succession. This result is in accordance with several studies carried out in alpine environments across the Alps (Albrecht et al. 2010; Wirth et al. 2011), Scandinavia (Elberling and Olesen 1999; Totland 2003; Lázaro et al. 2013) and Patagonia (Devoto et al. 2005). On the other hand, *S. bryoides* showed a diversified insect assemblage with pollinators, parasitoids, predators, and opportunists unevenly distributed among successional stages.

The abundance, but also the the taxonomic and the functional diversity, of flower-visiting insects increased along the succession. This pattern was consistent with the trends emerged in previous studies for pollinators (Albrecht et al. 2010) and epigean arthropod communities (Kaufmann 2001; Gobbi et al. 2006; Gobbi et al. 2010; Vater and Matthews 2013) along other alpine glacier forelands. Furthermore, the observed patterns in flower-visiting insects resembled the changes in plant community diversity. Therefore, this result may indicate the presence of common and related mechanisms in the assembly of both plant and insect communities (Losapio et al. 2015), as well as the direct role played by plant succession in structuring the flower-visiting insect community.

Indeed, the observed change in plant community structure was the main source of variation for the distribution and abundance of flower-visiting insects along the succession. The most influential ecological factors were related to floral resource abundance, such as the

382 number of *L. alpina* inflorescences and *S. bryoides* cover, and to plant community diversity
383 and biomass, such as total plant cover and the density of insect-pollinated plants. These
384 results highlight the relevance of biotic interactions, in contrast with the generally minor role
385 played by abiotic conditions, for structuring communities along a glacier foreland succession.
386 Furthermore, different flower-visiting insect groups were differently related to
387 successional changes of the vegetation. This pattern suggests idiosyncratic changes of plant–
388 insect interactions along the successional gradient. Pollinators of *S. bryoides* were associated
389 with the density of insect-pollinated plants and to *S. bryoides* cover. As a consequence, the
390 abundance of floral resources at both community and population levels affected the
391 distribution and the abundance of *S. bryoides* pollinators. Other studies linking the abundance
392 of floral resources to the activity of pollinatorsa showed that insects are strongly influenced
393 by floral density (Hegland and Boeke 2006). However, pollinators of *L. alpina* were
394 ubiquitously distributed, with low species turnover along the succession and independently
395 from *L. alpina* abundance and plant community structure. A possible explanation is that *L.*
396 *alpina* pollinators were generalists as they might feed on different species, so they did not
397 respond to changes in *L. alpina* floral abundance (Hegland and Boeke 2006) nor to
398 successional change of the vegetation.
399 Parasitoids, predators and opportunists characterised late successional stages and were
400 mainly related to an increase in plant cover. This is probably due to positive bottom-up effects
401 of plant community biomass and diversity on the abundance (Gobbi et al. 2010) and diversity
402 (Scherber et al. 2010) of resources and prey, which, in turn, might increase the abundance and
403 diversity of predators, parasitoids and opportunists.
404 Overall, our results suggest that successional changes of the vegetation are direct and
405 indirect drivers of the insect community assembly. If plant diversity and biomass influence

406 higher trophic levels through bottom-up trophic cascades (Scherber et al. 2010), in turn
407 pollinators may influence plant reproductive output (Albrecht et al. 2012) and plant
408 interactions (Lázaro et al. 2014) through top-down controls.
409
410 **Plant breeding system**
411 Based on our bag experiments we can conclude that *L. alpina* is strictly cross-pollinated, i.e. it
412 relies on insects for reproduction, while *S. bryoides* is self-pollinated, i.e. it does not require
413 insects for reproduction. In line with arctic-alpine plant reproductive strategies outlined by
414 Molau (1993), *L. alpina* had a pollen-risker profile, with high outbreeding rates and
415 reproduction resources allocated to pollen production and dispersion. On the other hand, *S.*
416 *bryoides* is an early-flowering seed-risker with high autogamy rates, in spite of the
417 proterandry as a phenological mechanism to enhance heterogamy (Ladinig and Wagner
418 2007), and resources allocated to seed production and recruitment.
419 The reproductive output of both plant species varied along the successional gradient. As
420 *L. alpina* requires pollinators for reproduction, it is expected to interact mainly with
421 pollinators and to show a reproductive output linked to pollinators availability (Molau, 1993).
422 Vice versa, as *S. bryoides* was able to self-fertilization, it is expected to interact with other
423 insect groups then pollinators and to show a reproductive output that is not limited by
424 pollinator availability. Consistently, *L. alpina* mainly interacted with pollinators while *S.*
425 *bryoides* was also interacting with parasitoids, predators and opportunists. However, contrary
426 to our expectation, the reproductive output of *L. alpina* was not linked with pollinator
427 abundance. This suggests that the reproductive output of this obligate insect-pollinated
428 species may also be influenced by other factors then pollinator abundance, such as the
429 heterospecific flower density of neighbouring plant community (Lázaro et al. 2013) or

competitive and facilitative interactions among co-flowering species for pollinators (Hegland and Totland 2012). Surprisingly, there was a link between reproductive output of *S. bryoides* and pollinator abundance. This result suggests that, for this species, out-crossing took priority over selfing, with pollinator availability as an important factor for *S. bryoides* reproductive output.

Conclusion

Our study is one of the few attempts to analyse plant–insect community feedbacks along a successional gradient. Our approach offers a link between ecological interactions and community assembly, revealing the relationships among different levels of ecological organization. The key finding of our study is that structural changes of the vegetation during succession influenced the distribution of flower-visiting insects. Although flower-visiting insects would be able to cover the 1.2 km distance along the glacier foreland, the composition of insect assemblages changed with succession proceeding due to guild-specific responses of different insect functional groups to changes in plant community properties. Indeed, the flower-visiting insect assemblage was initially dominated by pollinators, whose distribution was related to the density and richness of insect-pollinated plants. With succession proceeding insect assemblages became more diversified, with parasitoids, predators and opportunists associated to the increase in plant cover and diversity. Overall, these findings provide an important step forward towards a mechanistic understanding of the dynamics underlying the assembly of interacting communities of different trophic levels. Our results suggests that the assembly of alpine plant and insect communities may mainly be driven by biotic interactions with inherent feedback effects, rather than only by harsh abiotic environmental conditions and stochastic events. Thus, we emphasise the importance of biotic interactions underlying

ecological processes in alpine ecosystems.

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582 **Tab. 1** Generalized linear models were used to test differences of ecological properties of the
583 plant communities among successional stages. The significance of the predictor variable was
584 assessed by comparing a model with and without the predictor.

Ecological index	χ^2	**df**	**p**
Plant cover	54.077	4	< 0.001
Shannon diversity	20.913	4	< 0.001
Shannon evenness	22.240	4	< 0.001
e.p.d.	22.737	4	< 0.001
e.p.r.	5.370	4	0.251

585

586 **Tab. 2** pCCA synthesis results considering both taxonomic and functional features (level) of
587 insect assemblages and as response variables abundance data of insects (i.e. number of
588 individuals) pooled per sampling unit. Response variables: Ant = Anthomyiidae; Mus =
589 Muscidae; Syr = Syrphidae; Stap = Staphylinidae; Icn = Ichneumonidae; Pol = Pollinators;
590 Pred = Predators; Opp = Opportunists; Phy = Phytophagous; Par = Parasitoids. Variance
591 indicate the percentage explained by explanatory variables. Shown are the most relevant
592 explanatory variables (explanatory): Cov = plant community cover; e.p.r. = insect-pollinated
593 plant richness; e.p.d. = insect-pollinated plant density; Leu = number of *L. alpina*
594 inflorescences; Sax= *S. bryoides* cover.
595

Leucanthemopsis alpina

Level	**Response**	**Variance [%]**	**Explanatory**
Taxonomic	Ant, Mus, Syr, Stap	28.9	e.p.r.+e.p.d.
Functional	Pol, Pred, Opp	40.6	Leu + Cov + e.p.r.

Saxifraga bryoides

Level	**Response**	**Variance [%]**	**Explanatory**
Taxonomic	Ant, Mus, Stap, Icn	59.0	e.p.d. + Cov
Functional	Pol, Pred, Opp, Phyt, Par	60.8	Sax + e.p.d. + Cov

596
597

598 **Fig. 1** Biplot diagram showing the two principal axes of the plant community PCA based on
599 the relative cover values of all plant species. Plots and successional stages are represented
600 with different symbols, variable vectors (cover, Shannon diversity, evenness, e.p.d. and e.p.r.
601 indices) with arrows and successional stages with diamonds, connected by a line which
602 demonstrates their ranking according to the topographical sequence of the study sites. PC =
603 Principal component. The coordinate of the arrows relative to the top and right axes indicate
604 the correlation between variables and the first and the second principal axis, respectively.

605 **Fig. 2** Ecological indices of the flower-visiting insect community along the successional
606 gradient. Number of insect visits **(a, c)**, taxonomic (dashed lines with circles) and functional
607 (solid lines with triangles) Shannon diversity index **(b, d)** for *L. alpina* **(a-b)** and *S. bryoides*
608 **(c-d)**. Data were pooled for each successional stage.

Fig. 3 pCCA of the *L. alpina* insect family abundance **(a)**. Variance was decomposed in 43.7% weather conditions, 28.9% explanatory variables, and 27.4% unexplained. For the first axis net explained variance was 34.0%. and for the second axis 11.7%. pCCA of *S. bryoides* insect family abundance **(b)**. Variance was decomposed in 21.5% weather conditions, 59.0% explanatory variables, and 19.5% unexplained. For the first axis net explained variance was 60.2% and for the second axis 9.5%. pCCA of the *L. alpina* insect functional roles abundance **(c)**. Variance was decomposed in 6.8% weather conditions, 40.6% explanatory variables, and 52.6% unexplained. The first axis explained 35.0% of the net variance and the second axis 8.6%. pCCA of *S. bryoides* insect functional roles abundance **(b).** Variance was decomposed in 10.6% weather conditions, 60.8% explanatory variables, and 28.6% unexplained. For the first axis net explained variance was 57.0% and for the second axis 9.9%. Left axes are scaled proportional to species eigenvalues, top and right axes are scaled proportional to the correlation between the axis and the explanatory variable. The coordinates on the pCCA axis 2 of four 'outlying' points (3 in panel c; 1 in panel d) were reduced to 3.5 (-3.5 when negative) in order to improve the readability of the diagrams. The coordinates of the arrows relative to the top and right axes indicate the correlation of the environmental variable mentioned above with the first and the second canonical axes. 'Environmental' explanatory variables: shan = plant community Shannon index; cov= plant cover; leu = *L. alpina* cover; sax= *S. bryoides* cover; epd = insect-pollinated plant density; epr = insect-pollinated plant relative richness; flowers= *S. bryoides flowers*. *Centroid* of categorical environmental variables (panel a and c): inf<10 = less than 10 inflorescences of *L. alpina*; inf20-25 = 20-25 inflorescences of *L. alpina*; inf>30 = more than 30 inflorescences of *L. alpina*; cov<50 = plant cover lower than 50%, cov>70 = plant cover higher than 50%.